EFFECT OF DIFFERENT TYPES OF SOLVENT ON EXTRACTION OF PHENOLIC COMPOUNDS FROM *Cosmos caudatus*

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ABSTRACT

The preliminary screening indicated that *Cosmos caudatus* had extremely high antioxidant capacity. The antioxidant activity of most of the plant produced is mainly due to the presence of phenolic compounds. Among the phytochemicals, phenolic compounds are the main contributor of antioxidant activity in plant extracts due to their higher value in total phenolic content. The purpose of this study is to investigate the effect of different types of solvent with 50%, 70% and 100% concentration of each solvent on extraction of phenolic compounds from *Cosmos caudatus.* The soxhlet extractor was used in this study. Total phenols in the extract was determine using Folin-Ciocalteu (FC) assay. From the results, 100% ethanolic extract showed the highest of total phenolic content with 15.61 mgGAE/g. However the antioxidant activity was only 14.15%. Meanwhile, 70% acetone extract exhibited the highest inhibition of DPPH. The value obtained was 7.77 mgAAE/g with the antioxidant activity of 84.78%. The polarity of the solvent affects the efficiency of the extraction, total phenolic content and antioxidant activity of the obtained extracts. Total phenolic content is not the only contributor to its antioxidant activity. The existence of other components in fresh extract such as enzymes and vitamin may directly react with free radicals in addition to polyphenolic compound. Further research is warranted to explore the individual or major polyphenolic groups and other bioactive compounds in the *Cosmos caudatus.*

ABSTRAK

Dalam pemeriksaan awal menunjukkan bahawa *Cosmos caudatus* mempunyai kapasiti antioksidan . Aktiviti antioksidan yang banyak dihasilkan dalam tumbuhan ini terutamanya disebabkan oleh kehadiran sebatian fenolik. Antara fitokimia, sebatian fenolik ialah penyumbang utama aktiviti antioksidan dalam ekstrak tumbuhan disebabkan nilai yang tinggi dalam jumlah kandungan fenolik. Tujuan kajian ini untuk menyiasat kesan bagi jenis pelarut yang berlainan dengan kepekatan 50%, 70% dan 100% bagi setiap pelarut untuk pengekstrakan sebatian fenolik daripada *Cosmos caudatus*. Kaedah pengekstrakan daripada soxhlet telah digunakan dalam eksperimen ini. Bagi menentukan jumlah fenol dalam ekstrak perlu menggunakan Folin Ciocalteu. Berdasarkan keputusan, 100% ekstrak ethanol menunjukkan kandungan fenolik yang paling tinggi sebanyak 15.61 mg GAE/ g. Walaubagaimanapun, aktiviti antioksida hanyalah 14.15% Manakala, 70% aseton ekstrak menunjukkan kandungan antioksidan yang tinggi. Nilai yang diperolehi ialah 7.77 mg AAE/g dengan kandungan antioksidan sebanyak 84.78%. Kekutuban pelarut mempengaruhi keberkesanan pengekstrakan, jumlah kandungan fenol dan aktiviti antioksidan untuk ekstrak yang diperolehi. Jumlah fenol bukan hanya penyumbang kepada aktivit antioksida. Kewujudan komponen lain di dalam ekstrak seperti enzim dan vitamin boleh terus bertindak dengan radikal bebas dalam penambahan kumpulan polifenolik. Kajian lanjut adalah wajar untuk meneroka kumpulan polifenolik yang utama atau individu sebatian bioaktif yang lain dalam *Cosmos caudatus*.

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LIST OF ABBREVIATIONS

UV- Vis	Ultra- violet visible spectroscopy
HPLC	High Performance Liquid Chromatography
DPPH	1,1- diphenyl-2- picrylhydrazyl
TPC	Total Phenolic Content
FRAP	Ferric Reducing Antioxidant Power
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
TFC	Total Flavonoid Content
FC	Folin Ciocalteu
GAE	Galic acid equivalent

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Plants are potential sources of natural antioxidants. They produce various antioxidative compounds to counteract reactive oxygen species in order to survive (Lu and Foo, 1995). For the consumption of raw vegetables they are considered as traditional healthy diet (Ong *et al*.,2004). Consistent intake of raw vegetables is believed can prevent degenerative diseases such as cancer, diabetes, hypertension and cardiovascular. In fact, it can decrease the sign of aging, and improving physical fitness (Mohamed *et al*., 2005). This diet is a rich source of antioxidant contents like phytochemicals, vitamins and enzymes and also the other minerals and fibers beneficial to health.

Antioxidant is a molecule that capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that can damage cells. In fact antioxidants can terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols (Sies *et al*., 1997).

Plant phenolics are commonly found in both edible and non-edible plants and also have been reported to have multiple biological effects including antioxidant activity. Mainly the antioxidant activity of phenolics is due to their redox properties which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The importance of natural phenolic compounds from plants materials is raising interest among scientists, food manufacturers, and consumers due to functional food with specific health effects (Loliger *et a*l., 1991).

Herbs can be eaten fresh as a vegetable like salad and ulam. These herbs are believed to be associated with antioxidant activities and have many beneficial effects and one of them is ulam raja and in scientific name is *Cosmos caudatus*.as shown in Figure 1.1 It is known as an aromatic herb come from tropical Central America and now widespread in almost all tropical regions. Its young leaves are often eaten raw with sambal belacan or coconut paste and normally are used as kerabu. Due to their unique taste and aroma,it is also used as an appetizer and food flavouring. Several antimutagen and antifungal compounds from ulam raja for example lutein (Ragasa *et al.,*2005).

Figure 1.1: *Cosmos caudatus*

In this study, other factors were standardized except for extraction solvent. Thus, no specific or appropriate extraction solvent is recommended for optimal recovery of total phenolic content from fresh sample matrix to the chemical structures phenolics from simple and free to conjugated and polymerized forms that might consequently affect their solubility behavior (Prior *et al*., 2005). For the selection of solvent systems for this study was made on the basis of their reported efficiency in extracting phenols and other antioxidant compounds from fresh sample matrix (Luthria *et al*., 2006; Sun *et al*., 2007; Alothman *et al*., 2009).

Natural antioxidants can obtain from the extraction of fruits or vegetables. For those who need the antioxidant in their bodies, they can get it through the fresh fruit when they eat. But in the food and pharmaceutical industries, the extract of antioxidants from fruits and vegetables is needed for their manufacturing process. The natural antioxidants can be extracted through the fruits, vegetables, and herbs. Extraction is used when to separate substances and the process of extracting the antioxidant can be done by different method of extraction. For example, it can be done by using solvent which is a desired substance dissolves in the extraction and the undesired substance does not dissolve. There are several ways to do in the extraction process such as soxhlet extraction, hydrodistillation, ultrasonic extraction and many more. Soxhlet extraction is one of the oldest method and most widely used approaches for conventional extraction of solid samples (Dingler's *et al*., 1879).

Nowadays, there are numerous techniques such as 1,1- diphenyl-2- picrylhydrazy (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP) assays that are available to evaluate plant phenolics and antioxidant activities (Anatolovich *et al*.,2002). This study is conducted to evaluate the phenolic compounds from *Cosmos caudatus* extract by using different types of solvents and also to investigate the relationship between the antioxidative activity and total phenolic content of the ulam raja extract.

1.2 PROBLEM STATEMENT

Extraction yield of total phenolic compounds and recovery of antioxidant compounds from plant materials are typically depending on different extraction method. Besides, the difference in polarities of extracting solvents might influence the solubility of chemical constituents in a sample and its extraction yield. Therefore, the selection of an appropriate solvent system is one of the most relevant steps to determine of total phenolic content and other antioxidant compound from a sample.

1.3 RESEARCH OBJECTIVES

The main objective of this research is to investigate the effect of different type of solvents on extraction of phenolic compounds from *Cosmos caudatus.*

1.4 SCOPE OF RESEARCH

The scopes of the research are:-

a) Investigating the effects of different types of solvents on the extractability of total phenolic content from *Cosmos caudatus.*The solvent used are acetone , ethanol , and distilled water.
b) Identifying the antioxidant activity of *Cosmos caudatus.*extract.
c) Demonstrating the relationship between phenolic compound and antioxidant activity of *Cosmos caudatus* extract.

1.5 RATIONALE AND SIGNIFICANCE

The rationale and significance in this study is high total phenolic compounds contain in *Cosmos caudatus* act as natural antioxidant and have many medical benefits. The most applicable and healthy way to improve the antioxidant level in the body is by consuming different naturally food resources that available and act as a natural supplement. The best solvent to extract phenolic compounds of *Cosmos caudatus* can promise high potential to be used in nutraceuticals or in food industry as natural preservatives as well.

CHAPTER 2

LITERATURE REVIEW

2.1 PLANT MATERIAL

Ulam raja or *Cosmos caudatus* is an aromatic herb. It came from tropical Central America and now spread in almost all tropical regions. Its young leaves are often eaten raw with chilli or coconut paste and are used in dishes. Due to their unique taste and aroma they are used as an appetiser and food flavouring. There are several bioactive components in ulam raja and according to Ragasa (2005) *Cosmos caudatus* has antimutagen and antifungal compounds. Protein and amino acid are compositions of ulam raja (Zanariah *et al*., 2005). *Cosmos caudatus* is recommended in the traditional medicine system especially for improving blood circulation.

2.2 POLARITY OF SOLVENTS OF *Cosmos caudatus*

Plants are potential sources of natural antioxidants. They produce various antioxidative compounds to counteract reactive oxygen species in order to survive (Lu and Foo, 1995). The recovery, yield and type of phenolics in an extract are influenced by the type and polarity of extracting solvents, time and temperature of extractions as well as physical characteristic of the samples (Naczk and Shahidi, 2006).

Solvent extraction is most frequently used technique for isolation of plant antioxidant compounds. However, the extract yields and resulting antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvent due to the presence of different antioxidant compounds of different chemical characteristics and polarities that may or may not be soluble in a particular solvent. Polar solvents are frequently for the recovery of phenols from a plant matrix. The most suitable of these solvents are hot or cold aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate (Bonoli *et al*., 2009).

In addition, methanol and ethanol have been extensively used to extract antioxidant compounds from various plants and plant-based foods like fruits, vegetables and so on. According to Bonoli *et al* (2009) the maximum phenolic compounds were obtained from the mixtures of ethanol and acetone. In fact, it is important to evaluate and quantify effective antioxidant principles of medicinally or economically viable plant materials.

The polarity of the solvent for the different antioxidant compounds affects the efficiency of the extraction and the activity of the obtained extracts. For example water, methanol, ethanol, acetone, aqueous solutions solvents and ethyl acetate are commonly used as extraction solvents (Shui and Leong, 2006). For the certain complications arise when recovering phytochemical compounds from plant by products due to their high enzyme activity. However, drying the plant by- product before extraction, will immediately immersing the by- product in methanol (Arts and Hollman, 1998).

2.3 ANTIOXIDANT

An antioxidant in food is really important as it can protect human body from free radicals activity. It is also has capable of slowing or preventing the oxidation of other molecules. When electrons are transferred form a substance to an oxidizing agent, it called as oxidation reaction. Free radicals can be produced during the oxidation reactions, where the start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols.

Epidemiological studies have strongly suggested that diet plays an important role in the prevention of chronic diseases (Bauman *et al.,* 2004; Willet, 1995). Polyphenolics, thiols, carotenoids, tocopherols, and glucosinolates commonly found in fruits, vegetables and grains, provide chemoprotective effects to combat oxidative stress in the body and maintain balance between oxidants and antioxidants to improve human health (Adom and Liu, 2002; Dragsted *et al.,* 1993; Jia *et al.,* 1999; Wolfe *et al.,* 2003). An imbalance caused by excess oxidants leads to oxidative stress, resulting in damage to DNA and protein and increased risk of degenerative diseases such as cancer (Farombi *et al.,* 2004).

Consumption of fresh fruits and vegetables has been associated with reduced risk of coronary heart disease (CHD) (Bazzano *et al.,* 2003; Joshipura *et al*., 2001; Srinath Reddy and Katan, 2004), stroke (Gillman *et al*., 1995; Voko *et al.,* 2003), symptoms of chronic obstructive pulmonary disease (Fabricius and Lange, 2003; Liu *et al.,* 2004) and different types of cancer including breast and ovarian cancer (Duncan *et al.*, 2004) and colon cancer (Frydoonfar *et al.,* 2003). Polyphenolic compounds, widely distributed in higher plants, have been found to have potential health benefits that are believed to arise mainly from their antioxidant activity (Liu *et al*., 2003). There is considerable scientific and public interest in the important role than antioxidants may play in health care, such as by acting as cancer chemo preventive and anti-inflammatory agents and by reducing risk of cardiovascular mortality (Cos *et al*., 2004).

2.4 TOTAL PHENOLIC CONTENT

In organic chemistry phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) attached to an aromatic hydrocarbon group. The simplest of the class is phenol (C_6H_5OH) as shown as Figure 2.2.

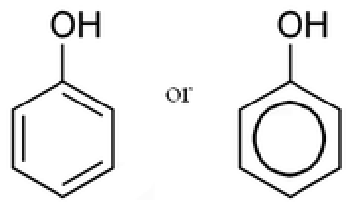

Figure 2.1: Structure of phenol

2.4.1 Phenolic Compound and Phenolic Acids

Phenolic compounds are essential for the growth and reproduction of plants and are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new high in recent years.

Besides that, plant phenolic compounds are diverse in structure but are characterized by hydroxylated aromatic rings for example flavan-3-ols. They are categorized as secondary metabolites, and their function in plants is often poorly understood. Many plant phenolic compounds are polymerized into larger molecules such as the proanthocyanidins and lignins. Furthermore, phenolic acids may occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxyfatty acids, sterols, and glucosides.

Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential as protective role through ingestion of fruits and vegetables, against oxidative damage diseases such as coronary heart disease, stroke, and cancers. Its compounds seem to be universally distributed in plants. They have been the subject of a great number of chemical, biological, agricultural, and medical studies. Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids.

2.5 THE CORRELATION BETWEEN PHENOLIC COMPOUND AND ANTIOXIDANT ACTIVITY

Phenolic compounds in plants are known to act as free radical scavengers. The antioxidant activity of most of the plant produce is mainly due to the presence of phenolic compounds (Skerget *et al*., 2005). Basically antioxidant mechanism of polyphenolic compounds is based on their hydrogen donating and metal ion chelating abilities. Among the phytochemicals, phenolic compounds are the main contributor of antioxidant activity in plant extracts due to their higher value in total phenolic content (Hodzic *et al*., 2009). In vegetables, phenolic compounds were reported to be dominated by glycocidic flavonols and hydroxycinnamic acids (Han *et al*., 2007).

Antioxidant treatments are thought to offset radical damage to biomolecules so slowing or the diseases by preventing oxidative stress. Phenolic compounds as major natural antioxidants of many fruits and vegetables are the focus of nutritional and therapeutic interest. Characterisation of the antioxidant activity of vegetables may also yield more insight into their functionality. Dietary antioxidants are necessary to cope with reactive oxidant species that could damage DNA, RNA and modify proteins. Antioxidants may inhibit the initiation or propagation of oxidation (Velioglu *et al*., 1998). Vegetable extracts with high antioxidant activity may also be useful for food preservation.

In fact, antioxidants are substances that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species, which include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non- radicals such as hydrogen peroxide. They scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Shi *et al.*, 2001). The most abundant antioxidants in fruits are polyphenols and Vitamin C, Vitamins A, B and E and carotenoids are present to a lesser extent in some fruits. These polyphenols mostly contain flavonoids are present mainly in ester and glycoside forms (Fleuriet and Macheix, 2003).

The correlation between phenolic compounds and antioxidant activity has been conducted in several studies. The antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, which are known to be potent as antioxidants (Wang *et al*., 1999). The effectiveness of phenolics and flavonoids as antioxidants is not only of their composition or relative amount but also by the degree of polymerization, concentration and interaction of their diverse chemical structures to the colorimetric assays. Thus, the higher levels of TPC and TFC do not necessarily correspond to the higher antioxidant responses (Parejo *et al*., 2002).

2.6 SOLVENT SYSTEM

Generally, for the extraction of polyphenols or other bioactive compounds from plant materials, water and organic solvents (ethanol, methanol, acetone, and diethyl ether) are used. Additionally, during the extraction process, the percent recovery depends mainly on the type of solvent and the extraction methods being adapted (Sun and Ho, 2005; Turkmen *et al*., 2006; Hayouni *et al*., 2007).

Solvents with low viscosity have low density and high diffusivity that allows them to easily diffuse into the pores of the plant materials to leach out the bioactive constituents (Naczk and Shahidi, 2006). For the change of solvent polarity, vapour pressure and viscosity of antioxidant compound that are being dissolved in the solvent also varies. As a result of this, the antioxidant activity of the extract observed also varies (Zhou and Yu, 2004; Turkmen *et al.*, 2006; Alothman *et al.*, 2009).

For optimal recovery of total phenolic content from fresh sample matrix to the diverse chemical structures of polyphenolics ranging from simple and free to conjugated and polymerized forms (lipophilic) might consequently affect their solubility behavior and there are no specific or appropriate extraction solvent is recommended (Prior *et al.*, 2005). The selection of solvent systems was made on the basis of the efficiency in extracting polyphenols and other antioxidant compounds from fresh sample matrix (Luthria *et al.*, 2006; Sun *et al.*, 2007; Alothman *et al.*, 2009).

2.7 SOXHLET EXTRACTOR

2.7.1 The Usage of Soxhlet Extractor

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet (Dingler's *et al.*, 1879). The method described by Franz von Soxhlet in is the most commonly used example of a semi-continuous method applied to extraction of lipids from foods. According to the Soxhlet's procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent usually hexane or petroleum ether under reflux in a special glassware. Four different extraction methods are possible without making any changes to the unit of soxhlet standard, soxhlet warm, hot extraction and continuous extraction. The system has an inert gas supply to avoid oxidation during extraction and to accelerate the evaporation and drying process even with high boiling point solvents (up to 150°C).

Besides that, soxhlet extractor is not limited to the extraction of lipids. Usually soxhlet extraction is only required where the desired compound has a limited solubility in a solvent and also the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent so a simple filtration can be used to separate the compound from the insoluble substance.

2.7.2 The Principle of Soxhlet Extractor

Solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent and then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will dissolve in the warm solvent. When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. Figure 2.2 shows example of a Soxhlet extractor.

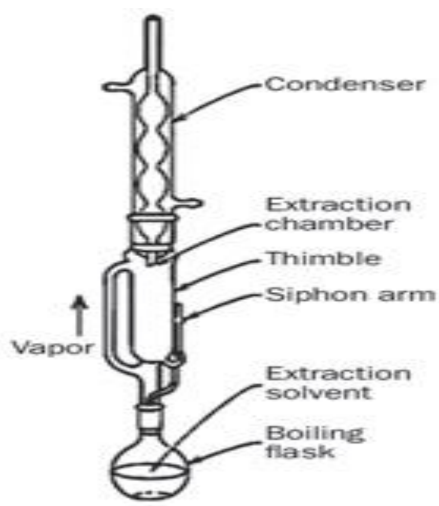

Figure 2.2: Soxhlet Extractor

2.7.3 The Advantages and Disadvantages of Soxhlet Extraction

2.7.3.1 Advantages of Soxhlet Extraction

The advantage of this system is that instead of many portions of warm solvent being passed through the sample just one batch of solvent is recycled. In fact the sample phase is always in contact with fresh solvent so enhancing the displacement of target compound from the matrix and the compound are not discomposed due to moderate extraction condition (Lee *et al*.,2000). After extraction the solvent is removed by a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded. Soxhlet extraction is one of the oldest method and most widely used approaches for conventional extraction of solid samples. It is the most conventional of all methods and consists of a simple distillation process repeated a number of times. Soxhlet extraction is straightforward and inexpensive (Luque de Castro *et.,al* 2004). In fact, it can maintain a relatively high extraction temperature with heat from the distillation flask and no filtration of the extract is required.

2.7.3.2 Disadvantages of Soxhlet Extraction

The disadvantage of this procedure is poor extraction of polar lipids. Agitation is not possible in the Soxhlet device. Besides that, a long time needed for the extraction process. The possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of the solvent for a long time. It also involved large volumes of solvents and exposed to the hazards of boiling solvents.